FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **204060US**

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): **09/763864**

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/JP00/04355	**30 JUNE 2000**	**30 JUNE 1999**

TITLE OF INVENTION
METHOD OF MAKING FERMENTATION PRODUCT

APPLICANT(S) FOR DO/EO/US
Masahide SATO, et al

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

Request for Consideration of Documents Cited in International Search Report
Notice of Priority
Drawings (8 sheets)
PCT/IB/304
PCT/IB/308

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/763864	PCT/JP00/04355	204060US

21. The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :		CALCULATIONS PTO USE ONLY
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO	$1,000.00	
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO	$860.00	
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$710.00	
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$690.00	
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)	$100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =		$860.00
Surcharge of **$130.00** for furnishing the oath or declaration later than ☒ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		$130.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	- 20 =	0	x $18.00	$0.00
Independent claims	- 3 =	0	x $80.00	$0.00
Multiple Dependent Claims **(check if applicable)**.			☐	$0.00
TOTAL OF ABOVE CALCULATIONS =				$990.00
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable)**.			☐	$0.00
SUBTOTAL =				$990.00
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).			+	$0.00
TOTAL NATIONAL FEE =				$990.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☐	$0.00
TOTAL FEES ENCLOSED =				$990.00
			Amount to be: refunded	$
			charged	$

☒ A check in the amount of **$990.00** to cover the above fees is enclosed.

☐ Please charge my Deposit Account No. in the amount of to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **15-0030** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

22850

Surinder Sachar
Registration No. 34,423

Surinder Sachar
SIGNATURE

Norman F. Oblon
NAME

24,618
REGISTRATION NUMBER

Feb. 28 2001
DATE

10 Rec'd PCT/PTO 23 MAY 2001

204060US0 PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF: :

SATO MASAHIDE ET AL : ATTN: NEW APPLICATION DIVISION

SERIAL NO: 09/763,864

FILED: FEBRUARY 28, 2001

FOR: METHOD OF MAKING FERMENTATION PRODUCTION PRODUCT :

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Prior to examination on the merits, please amend the above-identified application as follows:

IN THE SPECIFICATION

Page 1, line 2, replace the text in its entirety with the following:

--PROCESS FOR PRODUCING FERMENTATION PRODUCT--

IN THE CLAIMS

Please amend the claims as shown in the marked-up copy to read as follows:

--3. (Amended) A method according to claim 1, wherein said bioreactor is a fluidized bed type reactor comprising a fluidized bed section within which the immobilized microorganism is disposed, and a liquid circulating section for extracting a part of a fermentation liquid from a downstream side of said fluidized bed section and returning said part of fermentation liquid to an upstream side of said fluidized bed section.

5. (Amended) A method according to claim 1, wherein said immobilized microorganism is one which non-flocculent yeast is immobilized to a chitosan type immobilizing carrier.

6. (Amended) A method according to claim 1, wherein said non-flocculent yeast is a non-flocculent liquor yeast, and wherein said fermentation product is a liquor.

7. (Amended) A method according to claim 1, wherein said non-flocculent yeast is non-flocculent beer yeast, and wherein said fermentation product is a malt alcohol beverage.

8. (Amended) A malt alcohol beverage made by the method according to claim 1.--

PCT/JP95/02569

REMARKS

Claims 1-8 are active in the present application. The claims are amended to remove multiple dependencies. No new matter is added. An action on the merits and allowance of the claims is solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

Norman F. Oblon
Attorney of Record
Registration No. 24,618

Daniel J. Pereira, Ph.D.
Registration No. 45,518

09763854.052301

22850

(703) 413-3000
Fax #: (703) 413-2220
NFO:DJP\cct
I:\user\DJPER\204060US-pr.wpd

Marked-Up Copy
Serial No: _____
Amendment Filed on:
_____

IN THE SPECIFICATION

Please amend the specification as follows:

Page 1, line 2, replace the text in its entirety with the following:

--PROCESS FOR PRODUCING FERMENTATION PRODUCT--

IN THE CLAIMS

Please amend the claims as follows:

Page 1, line

--3. (Amended) A method according to claim 1 [or 2], wherein said bioreactor is a fluidized bed type reactor comprising a fluidized bed section within which the immobilized microorganism is disposed, and a liquid circulating section for extracting a part of a fermentation liquid from a downstream side of said fluidized bed section and returning said part of fermentation liquid to an upstream side of said fluidized bed section.

5. (Amended) A method according to [one of claims 1 to 4] claim 1, wherein said immobilized microorganism is one which non-flocculent yeast is immobilized to a chitosan type immobilizing carrier.

6. (Amended) A method according to [one of claims 1 to 5] claim 1, wherein said non-flocculent yeast is a non-flocculent liquor yeast, and wherein said fermentation product is a liquor.

7. (Amended) A method according to [one of claims 1 to 5] claim 1, wherein said non-flocculent yeast is non-flocculent beer yeast, and wherein said fermentation product is a

malt alcohol beverage.

8. (Amended) A malt alcohol beverage made by the method according to [one of claims 1 to 7] claim 1.--

09763864.052301

8/PRTS

09/763864
JC02 Rec'd PCT/PTO 28 FEB 2001
FP00-0129-00

09/763864 022801

DESCRIPTION

Method of Making Fermentation Product

Technical Field

The present invention relates to a method of making a fermentation product; and, more specifically, to a method of making a fermentation product by carrying out fermentation by use of a bioreactor within which an immobilized microorganism is disposed.

Background Art

As biotechnology advances, the making of fermentation products by use of bioreactors utilizing immobilized microorganisms is under study in the fields of brewing for malt alcohol beverages (beers), wines, sake, vinegar, soy sauce, and the like. When a bioreactor is used as such, the following are expected:

1) Since a high concentration of yeast is immobilized to carry out fermentation, the brewing is completed rapidly, so that the brewing period can be shortened, whereby the number of manufacturing tanks and the cost of construction can be lowered.

2) Since continuous fermentation is possible, it is unnecessary to charge and collect yeast.

Conventionally, when a beer or the like is manufactured by use of a bioreactor, however, the amount of amino acids and diacetyl (DA) has become greater, and the amount of ester has become smaller in the product as compared with a product

manufactured by a traditional batch fermentation method. As a result, the product obtained by use of the bioreactor has been problematic in that it is disadvantageous in flavor, and this problem has been remarkable in particular when the bioreactor is used for primary fermentation (main fermentation).

For overcoming such a problem, studies have conventionally been carried out. Japanese Patent Application Laid-Open Gazette No. HEI 7-123969 discloses a method in which a fermentation liquid is circulated in a continuous fermentation method using a fluidized bed type reactor. Though the consumption of amino acids in the fermentation process is ameliorated by this method, the amount of diacetyl which causes raw odor or immature odor is not lowered sufficiently, whereby the resulting product still has room for improvement in terms of flavor. Also, manufacturing methods in bioreactors including this method have been problematic in that the number of floating yeast cells upon the end (completion) of primary fermentation is small in general, the fermentation rate in the process of fermentation is unstable, and they are hard to control.

Meanwhile, it has conventionally been known that yeast for making a fermentation product such as beer is required to have flocculation ability (agglutinability) to a certain extent. Here, flocculation ability refers to a property in which yeast cells flocculate as a mass upon the end of

fermentation and are separated from the liquid so as to float up or sediment. If the flocculation ability is too high, then the flocculation occurs at an earlier stage, so that the contact between the fermentation liquid and yeast is broken, whereby fermentation becomes insufficient. If the flocculation ability is too low, by contrast, then yeast floats in the liquid for a long time, so that the amount of recovery of yeast decreases, which takes unnecessary time and labor for separating and collecting yeast by centrifuge or the like, for example. Therefore, it has conventionally been common knowledge to use yeast having a certain degree of flocculation ability (flocculent yeast) for making a fermentation product. Such flocculent yeast (agglutinative yeast) is used in the above-mentioned method disclosed in Japanese Patent Application Laid-Open Gazette No. HEI 7-123969 as well.

In view of the above-mentioned problems in the prior art, it is an object of the present invention to provide a method in which, when making a fermentation product by use of a bioreactor utilizing an immobilized microorganism, the fermentation rate in the fermentation process can be held constant, and the number of floating yeast cells upon the end of fermentation can stably be maintained at a level higher than the conventional one, whereby, when making a malt alcohol beverage by use of a bioreactor in particular, the amount of diacetyl in the fermentation liquid and final

product can be lowered sufficiently and so forth, so as to improve the flavor of final product.

Disclosure of the Invention

The inventors have repeated diligent studies in order to achieve the above-mentioned object, and have found that non-flocculent yeast (nonagglutinative yeast) can be employed contrary to the conventional knowledge when using a bioreactor utilizing an immobilized microorganism, since it is not necessary to separate and collect yeast, so that the fermentation rate in the fermentation process becomes constant, the number of floating yeast cells upon the end of fermentation is stably maintained at a level further preferable for secondary fermentation (after-fermentation), the amount of diacetyl in the fermentation liquid and final product sufficiently decreases in the case using such a bioreactor for primary fermentation of a malt alcohol beverage in particular, and so forth, whereby the flavor of product improves. Thus, the present invention has been accomplished.

Namely, the method of making a fermentation product in accordance with the present invention is a method of making a fermentation product by using a bioreactor within which an immobilized microorganism is disposed, wherein non-flocculent yeast is used as the microorganism.

The malt alcohol beverage of the present invention is made by the method of the present invention.

Brief Description of the Drawings

Fig. 1 is a schematic diagram of an example of fluidized bed type reactor suitable for the present invention;

Fig. 2 is a graph showing relationships between the batch number of fermentation times (the number of continuous fermentation processes) and the number of floating yeast cells;

Fig. 3 is a graph showing relationships between the batch number of fermentation times and the amount of extract consumption;

Fig. 4 is a graph showing relationships between the batch number of fermentation times and the number of floating yeast cells;

Fig. 5 is a graph showing relationships between the batch number of fermentation times and the amount of diacetyl;

Fig. 6 is a graph showing a relationship between the batch number of fermentation times and the amount of extract consumption;

Fig. 7 is a graph showing a relationship between the batch number of fermentation times and the number of floating yeast cells; and

Fig. 8 is a graph showing a relationship between the batch number of fermentation times and the amount of diacetyl.

Best Modes for Carrying Out the Invention

In the following, preferred embodiments of the present invention will be explained in detail.

The present invention is a method of making a fermentation product by carrying out fermentation by using a bioreactor within which an immobilized microorganism is disposed, wherein non-flocculent yeast is used as the microorganism.

First, the yeast and its immobilizing carrier in accordance with the present invention will be explained. Any yeast can be used in the present invention as long as it is non-flocculent as will be explained later, and non-flocculent ones are selected from yeast species corresponding to the aimed fermentation product. For example, as yeast used for making a liquor, non-flocculent ones are selected from so-called liquor yeast species which produce alcohol, carbonic acid gas, and the like by metabolizing a brewing material liquid. Specifically, non-flocculent ones are selected from Saccharomyces cerevisiae, Saccharomyces uvarum, and the like. Examples of such liquor yeast include non-flocculent beer yeast, non-flocculent wine yeast, and non-flocculent sake yeast. For example, non-flocculent beer yeast may be used so as to make a malt alcohol beverage such as beer.

Here, flocculation ability refers to a property in which yeast cells dispersed in a fermentation process flocculate (aggregate) so as to attach and bind to each other at cell surfaces, thereby forming a floc. Bottom fermentation yeast species used for normal beer production include strains which

tend to be rapidly separable from within a fermentation liquid by flocculating (aggregating) their cells to form a floc, and strains which are hard to flocculate and are likely to disperse and float for a relatively long period of time. The former are referred to as "flocculent yeast," whereas the latter are referred to as "non-flocculent yeast (also known as dust-like yeast)."

While it has been reported that such flocculation ability of yeast is essentially a genetic characteristic of yeast itself and is controlled by Lg-FLO 1 gene existing at a position corresponding to the VIII chromosome of Saccharomyces cerevisiae (Japanese Patent Application Laid-Open Gazette No. HEI 8-266287), it may also be influenced by water for brewing, materials, malt compositions, malt aeration conditions, yeast culture conditions, fermentation vessels, handling of yeast, and the like, and the strength of flocculation ability may vary as the fermentation process proceeds. Thus, the strength of flocculation ability varies depending on yeast strains, and the same species of yeast may include non-flocculent and flocculent ones depending on strains. Therefore, it is preferred in the present invention that a non-flocculent strain be selected by the following method and the like, so as to use non-flocculent yeast derived from this strain. In particular, it is preferred to use such non-flocculent yeast alone.

An example of methods for measuring the flocculation

ability of yeast so as to select non-flocculent yeast to be used for the method of the present invention as such is one described in YEAST GENETICS: FUNDAMENTAL AND APPLIED ASPECTS, 205-224 (1983). Specifically, the following method is preferred.

Namely, 0.6 g of yeast (one separated by precipitation upon centrifuge at 3000 × g for 10 minutes at the time when fermentation is completed) is added to 20 ml of tap water, so as to yield a yeast suspension. To 9 ml of this yeast suspension, 1 ml of 0.5-M acetic acid buffer solution at pH 4.5 including 1500 ppm of calcium ion is added. The resulting mixture is hand-shaken up and down, for example, so as to uniformly stir it as a whole. Then, the mixture is left to stand still for 5 minutes at room temperature.

Thereafter, the degree of agglutination is visually inspected (by the naked eye), and is evaluated according to the following four criteria:

0: Non-flocculent (No boundary is seen between the liquid and floc by the naked eye, and neither flocculation (aggregation) nor sedimentation of yeast is observed.)

1: Weakly Flocculent (Though no boundary can be seen between the liquid and floc by the naked eye, flocculation or sedimentation of a part of yeast is observed.)

2: Mildly Flocculent (A boundary can be seen between the liquid and floc by naked eye, and flocculation or sedimentation of yeast is observed.)

3: Strongly Flocculent (Yeast substantially completely flocculates and sediments, and the supernatant becomes substantially completely clear.)

It is preferred in the present invention that a non-flocculent strain satisfying the non-flocculent condition (level 0) in the above-mentioned evaluation criteria be selected, and non-flocculent yeast derived from this strain be used. In particular, it is preferable to use such non-flocculent yeast alone.

Thus, contrary to the conventional knowledge, the method of the present invention uses non-flocculent yeast derived from the non-flocculent strain, so that yeast is fully prevented from flocculating within the bioreactor in the process of fermentation and thereby sedimenting and precipitating, whereby the fermentation rate is held constant, and the number of floating yeast cells upon the end of fermentation is stably maintained at a level higher than that in the case where conventional flocculent yeast is used. In the case where the method of the present invention is employed in primary fermentation of a malt alcohol beverage in particular, the amount of diacetyl in the fermentation liquid upon the end of primary fermentation is sufficiently lowered since the number of floating yeast cells upon the end of primary fermentation improves, and diacetyl is further efficiently reduced in secondary fermentation, so that the amount of diacetyl in the final product sufficiently

decreases, thus lowering the resulting immature odor component, whereby the flavor of product improves.

Without being restricted in particular, various kinds of carriers can be used as a carrier for immobilizing the above-mentioned non-flocculent yeast. In particular, carriers comprising chitin-chitosan, alginic acid, carrageenan are preferable. Among others, chitosan type beads (carriers made from chitin-chitosan obtained by actylation of chitosan) are preferable. Since chitosan type beads are hydrophilic and porous, carbonic acid gas is easily let out therefrom. Also, they are hard to wear, and their fluidity is favorable since their density approximates that of the material liquid. Further, the chitosan type beads can hold a large amount of microorganisms, thereby tending to shorten the fermentation time more. Also, since microorganisms are adsorbed and immobilized by the chitosan type beads relatively mildly, their growth and desorption become easier, and dead cells are kept from continuously existing within the carrier as in a collective carrier.

Any method can be used for the sterilization carried out before immobilizing non-flocculent yeast onto such a carrier. Preferred are high-pressure sterilization method, sterilization method using caustic soda, sterilization method using steam, and the like. Also, the method of immobilizing non-flocculent yeast onto the carrier is not restricted in particular. While its examples include one

in which the carrier is added to a yeast suspension, and then the mixture is stirred or the liquid is circulated, other known methods may be used as well.

The bioreactor in accordance with the present invention will now be explained. The bioreactor in accordance with the present invention is one within which the immobilized microorganism (non-flocculent yeast immobilized to the carrier) is disposed, and the material liquid and immobilized microorganism come into contact with each other, whereby fermentation is carried out. Examples of such a bioreactor include, in terms of their types, complete mixed vessel type reactors, packed bed type reactors, film type reactors, fluidized bed type reactors, and lateral reactors. For fermentation in which alcohol and carbonic acid gas are generated upon metabolizing a brewing material, such as primary fermentation of malt alcohol beverages, it is preferable to use fluidized bed type reactors which easily let the gas out of the system.

Preferably, such a fluidized bed type reactor comprises a fluidized bed section within which an immobilized microorganism is disposed, and a liquid circulating section for extracting a part of the fermentation liquid from the downstream side of the fluidized bed section and returning it to the upstream side of the fluidized bed section. Fig. 1 is a schematic diagram showing an example of the fluidized bed type reactor suitable for the present invention.

The fluidized bed type reactor 1 shown in Fig. 1 comprises a reaction tank 2 and a liquid circulating section 3. The reaction tank 2 is constituted, successively from the upstream side thereof, by a rectifying section 4, a fluidized bed section 5, and an empty tube section 6, whereas the downstream end portion of the reaction tank 2 is provided with a gas outlet section 7. The liquid circulating section 3 is constituted by a pipe 31 connected to the downstream side of the fluidized bed section 5 and to the upstream side of the fluidized bed section 5 (to the rectifying section 4 in Fig. 1), a pump 32, and a valve 33. Further, the pipe 31 branches off on its way, so as to connect with a product tank 8 by way of a valve 34. Also, a material liquid tank 9 is connected to the upstream end portion of the reaction tank 2 by way of a pipe 92 having a pump 91.

The fluidized bed section 5 is a section within which the carrier having immobilized the microorganism is disposed, whereas the liquid circulating section 3 is a section for extracting from the downstream side of the fluidized bed section 5 a part of the fermentation liquid (material liquid) supplied to the reaction tank 2 and returning it to the upstream side of the fluidized bed section 5 of the reaction tank 2. In the reactor 1 shown in Fig. 1, the extracted fermentation liquid (material liquid) is returned into the reaction tank 2 from the rectifying section 4, where the flow of introduced liquid is rectified. The empty tube

section 6 is a section where the carbonic acid gas and fermentation liquid generated during fermentation are separated from each other as a gas and a liquid; and specifically is the part from the liquid surface to the gas outlet section 7 in the upper portion of the reaction tank 2. The gas such as carbonic acid gas isolated in the empty tube section 6 is let out of the vessel by way of the gas outlet section 7.

In the fluidized bed type reactor 1 shown in Fig. 1, the carrier having immobilized the microorganism is disposed in the fluidized bed section 5, and the material liquid is supplied to the rectifying section 4 from the material liquid tank 9 by use of the pump 91, so as to carry out fermentation. Then, a part of the fermentation liquid (material liquid) is extracted from the downstream side of the fluidized bed section 5 by use of the pump 32 of liquid circulating section 3 and is returned to the upstream side of the fluidized bed section 5 (to the rectifying section 4 in Fig. 4) in the reaction tank 2, whereby fermentation is carried out while forming a fluidized bed. The carbonic acid gas generated during fermentation is let out of the vessel via the gas outlet section 7 by way of the empty tube section 6 without staying within the fluidized bed, since the fermentation liquid is circulated and so forth. While the liquid space velocity (linear velocity of fluid per unit volume of fluidized bed) can be changed depending on the density of

the carrier having immobilized the microorganism, it is preferably 1 to 20 cm/min, more preferably 1 to 12 cm/min.

It is preferred in the fluidized bed type reactor 1 shown in Fig. 1 that, secondary fermentation is completed, the fermented liquid including a fermentation product be taken out from the reactor 1 into the product tank 8 by way of the pump 32 and valve 34, and a new material liquid be supplied from the material liquid tank 9 to the reactor 1 by use of the pump 91, so as to repeatedly carry out the fermentation. Namely, after the end of fermentation, the circulation is stopped and, concurrently with or immediately after the fermented liquid is extracted out of the reactor 1, a new material liquid is supplied to the reactor 1, so as to repeatedly carry out the fermentation.

While the reactor 1 can be operated by any of batch type, repeated batch type, and continuous type methods, repeated batch type fermentation is preferable for yielding a product having a better flavor in a short time. The repeated batch type operation tends to yield a better flavor since the microorganism is grown and renewed during fermentation, while the physiological state and growth period of microorganic cells, the microorganic cell distribution within the reactor 1, and the like are similar to those in the batch type operation, which is a traditional method of making liquors.

Any material for making a fermentation product may be

used as long as it is suitable for fermentation caused by the nonagglutinative yeast employed, and known materials can be used at will. For example, malts, fruit juices, sugar liquids, cereal saccharified liquids, and the like are normally used alone or in combination as appropriate in the making of liquors. Also, appropriate nutrients and the like may be added thereto when necessary.

Fermentation conditions are not different from known conditions in essence. For example, the fermentation temperature is normally 15℃ or lower, preferably 8 to 10℃, in the case of brewing malt alcohol beverages; whereas it is normally 20℃ or lower, preferably 15 to 20℃, in the case of brewing wines.

Examples of fermentation products which can be made by the method of the present invention include products in various brewing fields such as malt alcohol beverages, wines, sake, vinegar, and soy sauce, among which liquors such as malt alcohol beverages, wines, and sake are preferred. In particular, malt alcohol beverages such as beer are preferable since their flavor is improved by the present invention.

Examples

In the following, the contents of the present invention will be explained in more detail with reference to Examples and Comparative Examples. However, the present invention is not restricted by these Examples at all.

In the following Examples and Comparative Examples, flocculent strains (A-1, A-2) and non-flocculent strains (NA-1, NA-2, NA-3, NA-4, NA-5) selected, according to the above-mentioned methods and criteria, from beer yeast (Saccharomyces cervisiae or the like) actually used in the site of manufacture were used. Table 1 shows the results of evaluation of their flocculation ability. For reference, the results of similar evaluation of flocculation ability concerning type cultures NCYC-No. 203 and NCYC-No. 985 in NCYC (National Collection of Yeast Cultures (United Kingdom)) are also shown in Table 1.

TABLE 1

RESULT OF FLOCCULATION ABILITY EVALUATION FOR BEERYEAST		
YEAST		RESULT
STRAINS USED IN THIS TEST	A-1	2
	A-2	2
	NA-1	0
	NA-2	0
	NA-3	0
	NA-4	0
	NA-5	0
TYPE CULTURE	NCYC-No.203	2
	NCYC-No.985	0

Examples 1 and 2 and Comparative Example 1

Using the fluidized bed type bioreactor (total volume: 180 ml) shown in Fig. 1, the primary fermentation was carried out by the above-mentioned repeated batch fermentation (repeated batch type) under the following conditions. Then, thus obtained fermentation liquid was subjected to a floating yeast cell counting test which will be explained later.

Primary Fermentation Conditions

Carrier: chitosan type beads (Chitopearl HP manufactured by Fuji Spinning Co., Ltd.)

Scale: 50 ml of carrier, 100 ml of wort

Strains Used: A-1 (Comparative Example 1), NA-1 (Example 1), NA-2 (Example 2)

Yeast Immobilizing Method: Yeast is immobilized as being brought into contact with the carrier for 2 days while being circulated according to a conventional method (2.4 g of sludge yeast with respect to 50 ml of carrier).

Flow Rate: about 1 ml/min

Fermentation temperature: constantly 8°C

Fermentation Time: 48 hours/batch

Batch number of Fermentation times: 10

Wort Used: wort (malt) adjusted to yield a sugar index of 11% Plato.

Floating Yeast Cell Counting Test

After the end of each primary fermentation process, the number of floating yeast cells in the fermentation liquid was counted by use of a Thoma blood cell counting plate. The obtained results are shown in Table 2 and Fig. 2. Here, first several repeated fermentation processes were excluded from the counting since they correspond to the acclimatization period of yeast in the carrier (ditto in the following).

As can be seen from the results shown in Table 2 and

Fig. 2, when non-flocculent strains were used under the above-mentioned conditions, the number of floating yeast cells upon the end of fermentation was stably held at 20 to 40 million cells/ml, which was high and closer to that in normal fermentation. When the flocculent strain was used, by contrast, yeast was seen to sediment onto the upper part of carrier in the last half of fermentation, and the number of floating yeast cells upon the end of fermentation was 10 million cells/ml or less until the seventh fermentation process.

Here, when the number of yeast cells in the carrier was measured after 10 fermentation times, it was about 10^9 cells/1 ml of carrier in each of the three strains, whereby it was verified that the number of immobilized yeast cells tolerable for practical use in a primary fermentation bioreactor was achieved in the non-flocculent strains as in the flocculent strain. When the state of yeast immobilized to the carrier was observed with an electronic microscope, it was verified that the non-flocculent strains were sufficiently immobilized in the chitosan type beads as with the flocculent strain. When the ratio of dead cells in and out of the carrier was investigated after 10 fermentation times, it was 10% or less in each of the three strains, whereby there was no problem.

TABLE 2

NUMBER OF FERMENTATION TIMES	NUMBER OF FLOATING YEAST CELLS UPON THE END OF PRIMARY FERMENTATION (UNIT: $\times 10^6$ cells/ml)							
	1	2	3	4	5	6	7	AVERAGE
NA-1	35	32	33	44	30	36	42	36.0
NA-2	26	32	32	34	14	25	22	26.4
A-1	5	7	9	5	8	7	11	7.4

Examples 3 and 4 and Comparative Example 2

Using the fluidized bed type bioreactor (total volume: 20 liters) shown in Fig. 1, the primary fermentation process was carried out by the above-mentioned repeated batch fermentation (repeated batch type) under the following conditions. Then, thus obtained fermentation liquid was subjected to a fermentative property test, a floating yeast cell counting test, and a diacetyl generated amount test which will be explained later.

Primary Fermentation Conditions

Carrier: chitosan type beads (Chitopearl HP manufactured by Fuji Spinning Co., Ltd.)

Scale: 6 L of carrier, 8 L of wort

Strains Used: A-2 (Comparative Example 1), NA-3 (Example 3), NA-4 (Example 4)

Yeast Immobilizing Method: Yeast is immobilized as being brought into contact with the carrier for 2 days while being circulated according to a conventional method (650 g of sludge yeast with respect to 6 L of carrier).

Liquid Space Velocity: 6 to 12 cm/min

Fermentation temperature: constantly 8°C

Fermentation Time: 48 hours/batch

Batch number of Fermentation times: 7

Wort Used: wort adjusted to yield a sugar index of 11% Plato.

Fermentative Property Test

In each primary fermentation process, the amount of consumption of extract during 24 hours after starting fermentation determined by a vibration type densitometer (DMA58, manufactured by Anton Paar GmbH) was used as an index for fermentation rate comparison. Thus obtained results are shown in Table 3 and Fig. 3.

As can be seen from the results shown in Table 3 and Fig. 3, the fermentation rate converged to substantially a constant value (about 6.7%/24 hr) at the first process and later in the case where the non-flocculent strains were used. The primary fermentation was completed in about 2 days. When the non-flocculent strains were used, no yeast was seen to precipitate in the reactor.

In the case where the flocculent strain was used, the flocculated and sedimented yeast tended to precipitate within the reactor vessel, so that the amount of yeast involved in fermentation increased, thereby accelerating the fermentation rate and making it inconstant. Here, the fermentation rate was slower in the third fermentation

process than in the second fermentation process in the case where the flocculent strain was used, because of the fact that the precipitated yeast was removed by exchanging malts in a short period of time therebetween. Thus, the fermentation rate was hard to control when the flocculent strain was used, and it was necessary to carry out processing such as elimination of precipitated yeast in order to stabilize the fermentation rate.

TABLE 3

	AMOUNT OF EXTRACT CONSUMPTION IN 24HR AFTER STARTING FERMENTATION (%)				
NUMBER OF FERMENTATION TIMES	1	2	3	4	AVERAGE
NA-3	6.4	7.0	7.2	6.8	6.8
NA-4	5.9	6.7	6.8	6.6	6.5
A-2*1	8.2	9.6	8.1	8.5	8.6

*1: In A-2, the inside of column was washed with wort (malt) between the second and third processes, so as to control the fermentation rate.

Floating Yeast Cell Counting Test

After the end of each fermentation process, the number of floating yeast cells in the fermentation liquid was counted in the same manner as Example 1. Thus obtained results are shown in Table 4 and Fig. 4.

As can be seen from the results shown in Table 4 and Fig. 4, when non-flocculent strains were used under the above-mentioned conditions, the number of floating yeast

cells upon the end of fermentation was stably held at high levels, i.e., 23 to 39 million cells/ml in NA-3, and 18 to 33 million cells/ml in NA-4. When the flocculent strain was used, by contrast, the number was 5 to 31 million cells/ml, and yeast was seen to sediment within the reactor in the last half of repeated batch fermentation, and the number of floating yeast cells upon the end of fermentation greatly fluctuated under the influence thereof.

TABLE 4

	NUMBER OF FLOATING YEAST CELLS UPON THE END OF PRIMARY FERMENTATION(10^6cells/ml)				
NUMBER OF FERMENTATION TIMES	1	2	3	4	AVERAGE
NA-3	24	23	39	28	28.5
NA-4	29	33	30	18	27.5
A-2	5	12	25	31	18.3

Diacetyl Generated Amount Test

After the end of each primary fermentation process, the amount of generation of diacetyl (DA) in the fermentation liquid was measured by a gas chromatography (GC-14B, manufactured by Shimadzu Corp.). Thus obtained results are shown in Table 5 and Fig. 5.

As can be seen from the results shown in Table 5 and Fig. 5, the amount of generation of diacetyl upon the end of primary fermentation was stably maintained at a low level of 0.3 to 0.6 ppm when the non-flocculent strains were used.

When the flocculent strain was used, by contrast, the amount was 0.4 to 1.3 ppm. It was seen that the amount of generation of diacetyl when the non-flocculent strains were used was about half that when the flocculent strains were used and was stably held at this low level.

TABLE 5

	AMOUNT OF DA UPON THE END OF PRIMARY FERMENTATION(ppm)							
NUMBER OF FERMENTATION TIMES	1	2	3	4	5	6	7	AVERAGE *2
NA-3	0.42	0.36	0.36	0.33	0.60	0.38	0.41	0.41
NA-4	0.55	0.49	0.39	0.42	0.37	0.44	0.42	0.44
A-2	0.53	0.42	0.98	0.49	1.27	0.90	0.78	0.77

*2: normal level: about 0.4 ppm

Example 5

Using the fluidized bed type bioreactor (total volume: 450 liters) shown in Fig. 1, the primary fermentation process was carried out by the above-mentioned repeated batch fermentation (repeated batch type) under the following conditions. Then, thus obtained fermentation liquid was subjected to a fermentative property test, a floating yeast cell counting test, and a diacetyl generated amount test by the same methods as mentioned above. Further, in this example, a flavor component test and an organoleptic test were carried out for the final product.

Primary Fermentation Conditions

Carrier: chitosan type beads (Chitopearl HP manufactured by Fuji Spinning Co., Ltd.)

Scale: 120 L of carrier, 170 L of wort

Strains Used: NA-5 (non-flocculent strain)

Yeast Immobilizing Method: About 13 kg of sludge yeast were immobilized as being brought into contact with 120L of the carrier while being circulated according to a conventional method as in Examples 3 and 4.

Liquid Space Velocity: 6 to 12 cm/min

Fermentation temperature: constantly 8℃

Fermentation Time: 48 hours/batch

Batch number of Fermentation times: 9

Wort Used: wort adjusted to yield a sugar index of 11% Plato.

Secondary Fermentation Conditions

Scale: 30 L

Period: 4 weeks

Secondary Fermentation Temperature: 8 to 0℃

In the fermentative property test of this example, as can be seen from the results shown in Table 6 and Fig. 6, the fermentation rate after the completion of adaptation period became substantially a constant value (average value of about 5.8%/24 hr). The precipitation of yeast within the bioreactor, which is seen when flocculent strains are used, was not observed in this case as well.

TABLE 6

NUMBER OF FERMENTATION TIMES	1	2	3	4	5	6	7	8	9	AVERAGE
AMOUNT OF EXTRACT CONSUMPTION (%/24HR)	5.6	5.8	5.4	5.8	5.3	6.2	5.7	6.7	6.0	5.8

In the floating yeast cell counting test of this example, as can be seen from the results shown in Table 7 and Fig. 7, the number was at a level of 10 to 20 million cells/ml (with an average value of about 15 million cells/ml), which was slightly lower than that in other examples using other smaller scale bioreactors, but it was possible for the number to be stably held at a level higher than a common level (3 to 10 million cells/ml) of the case where the flocculent strain was used in the 80-liter-scale bioreactor.

TABLE 7

NUMBER OF FERMENTATION TIMES	1	2	3	4	5	6	7	8	9	AVERAGE
NUMBER OF FLOATING YEAST CELLS ($\times 10^6$cells/ml)	14	17	17	21	13	10	11	11	18	14.6

In the diacetyl generated amount test in this example, as can be seen from the results shown in Table 8 and Fig. 8, the amount of diacetyl generation upon the end of primary fermentation was 0.5 to 0.7 ppm (with an average value of

about 5.8 ppm).

TABLE 8

NUMBER OF FERMENTATION TIMES	1	2	3	4	5	6	7	8	9	AVERAGE
DA(ppm)	0.50	0.48	0.50	0.54	0.67	0.52	0.52	0.50	0.64	0.54

The beer having completed the primary fermentation in the bioreactor was subsequently subjected to secondary fermentation for 4 weeks at a scale of 30 liters. Thereafter, analyses of low volatile components (L.V.C.) and diacetyl, and an organoleptic test were carried out for the final product beer. The analysis of low volatile components was carried out with a gas chromatography GC4A manufactured by Shimadzu Corp.

Table 9 shows the results of analyses of low volatile components and diacetyl concerning the final product beer obtained by use of the beer yielded by the ninth primary fermentation. As can be seen from the results shown in Table 9, the final product beer obtained by this example was characterized in that the amount of acetate was higher than the analyzed value of that in a standard beer, whereas the amount of diacetyl, which might become problematic in bioreactor fermentation in particular, was reduced and lowered to 0.02 ppm, which was at a level hardly detectable

organoleptically.

The results of a drinking test for the final product beer obtained in this example was not so different from those of conventional beers, and were favorable in general.

TABLE 9

ANALYSIS RESULT (ppm)	ACET-ALDEHYDE	ACETONE	ETHYL ACETATE	n-PrOH	i-BuOH	ISOAMYL ACETATE	i-AmOH	DA
REACTOR FERMENTATION	4.3	0.1	36	14.6	12.7	2.0	56	0.02
BEER STANDARD VALUE	1.4	0.6	20	9.2	7.1	1.5	50	0.01

Industrial Applicability

According to the method of the present invention, as explained in the foregoing, non-flocculent yeast is used contrary to the conventional knowledge when making a fermentation product by use of a bioreactor utilizing an immobilized microorganism, whereby the fermentation rate in the fermentation process can be held constant, and the number of floating yeast cells upon the end of fermentation can stably be maintained at a higher level.

When the method of the present invention is employed in the case of making a malt alcohol beverage by use of a bioreactor in particular, the amount of diacetyl in the fermentation liquid and final product can be lowered sufficiently and so forth, whereby the flavor of product can be improved.

EP 1 253 195 A1

CLAIMS

1. A method of making a fermentation product by using a bioreactor within which an immobilized microorganism is disposed, wherein non-flocculent yeast is used as said microorganism.

2. A method according to claim 1, wherein said non-flocculent yeast satisfies the following condition (a):

(a) when a yeast suspension is obtained by adding 0.6 g of yeast to 20 ml of tap water, 1 ml of 0.5-M acetic acid buffer solution at pH 4.5 including 1500 ppm of calcium ion is added to 9 ml of said yeast suspension, and the resulting mixture is uniformly stirred and is left to stand still for 5 minutes at room temperature, neither flocculation nor sedimentation of yeast is seen by the naked eye thereafter.

3. A method according to claim 1 or 2, wherein said bioreactor is a fluidized bed type reactor comprising a fluidized bed section within which the immobilized microorganism is disposed, and a liquid circulating section for extracting a part of a fermentation liquid from a downstream side of said fluidized bed section and returning said part of fermentation liquid to an upstream side of said fluidized bed section.

4. A method according to claim 3, wherein fermentation is carried out while forming a fluidized bed by extracting a part of a fermentation liquid from the downstream side of said fluidized bed section and returning

said part of fermentation liquid to the upstream side of said fluidized bed section, and said fermentation is repeatedly carried out by taking out thus obtained fermentation product from said reactor and supplying a new material liquid to said reactor.

5. A method according to one of claims 1 to 4, wherein said immobilized microorganism is one in which non-flocculent yeast is immobilized to a chitosan type immobilizing carrier.

6. A method according to one of claims 1 to 5, wherein said non-flocculent yeast is non-flocculent liquor yeast, and wherein said fermentation product is a liquor.

7. A method according to one of claims 1 to 5, wherein said non-flocculent yeast is non-flocculent beer yeast, and wherein said fermentation product is a malt alcohol beverage.

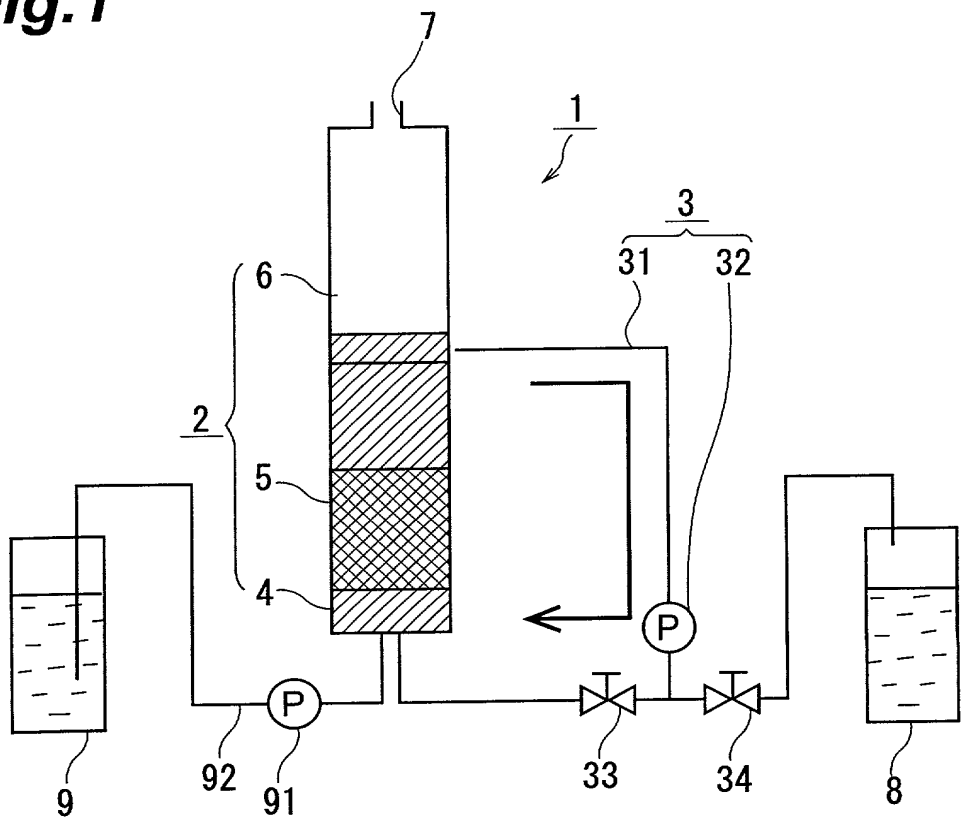
8. A malt alcohol beverage made by the method according to one of claims 1 to 7.

09753854.052301

ABSTRACT

In a method of making a fermentation product by using a bioreactor within which an immobilized microorganism is disposed, non-flocculent yeast is used as the microorganism.

Fig.1



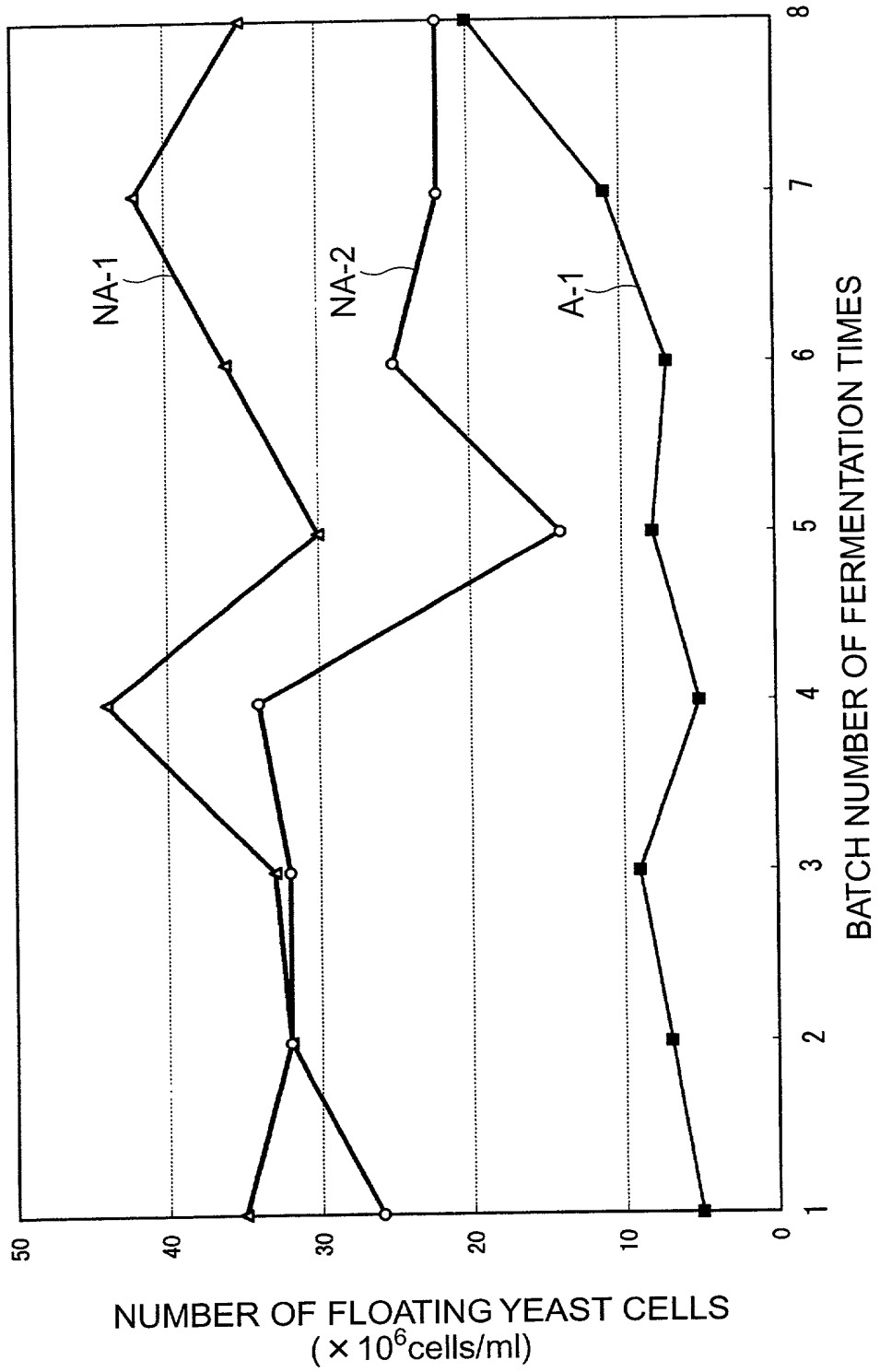

NUMBER OF FLOATING YEAST CELLS
UPON THE END OF PRIMARY FERMENTATION
NA-1
NA-2
A-1
50
40
30
20
10
0
1
2
3
4
5
6
7
8
BATCH NUMBER OF FERMENTATION TIMES
NUMBER OF FLOATING YEAST CELLS
(× 10^6cells/ml)

Fig.2

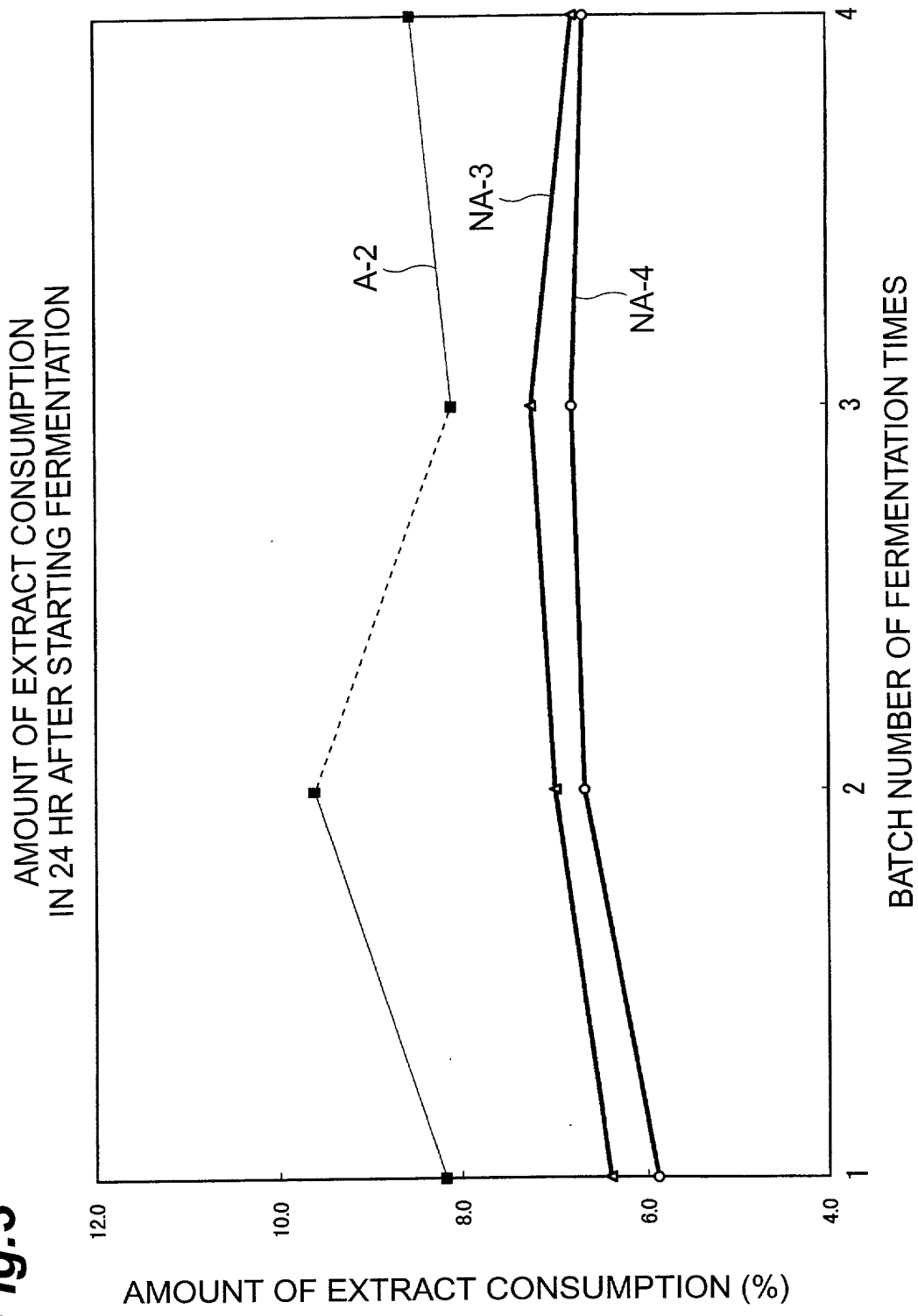
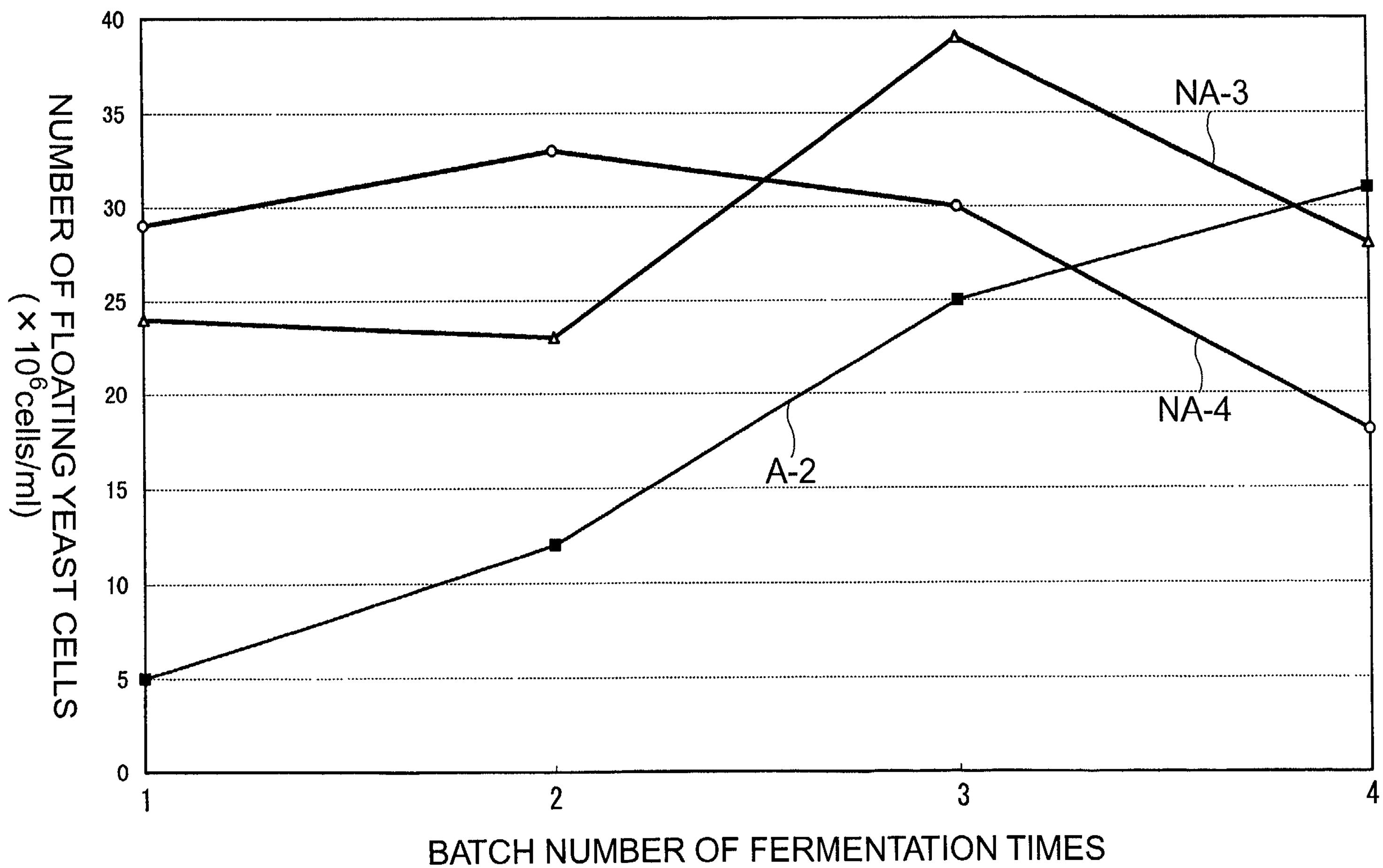

AMOUNT OF EXTRACT CONSUMPTION
IN 24 HR AFTER STARTING FERMENTATION
A-2
NA-3
NA-4
12.0
10.0
8.0
6.0
4.0
AMOUNT OF EXTRACT CONSUMPTION (%)
1
2
3
4
BATCH NUMBER OF FERMENTATION TIMES

Fig.3

Fig.4

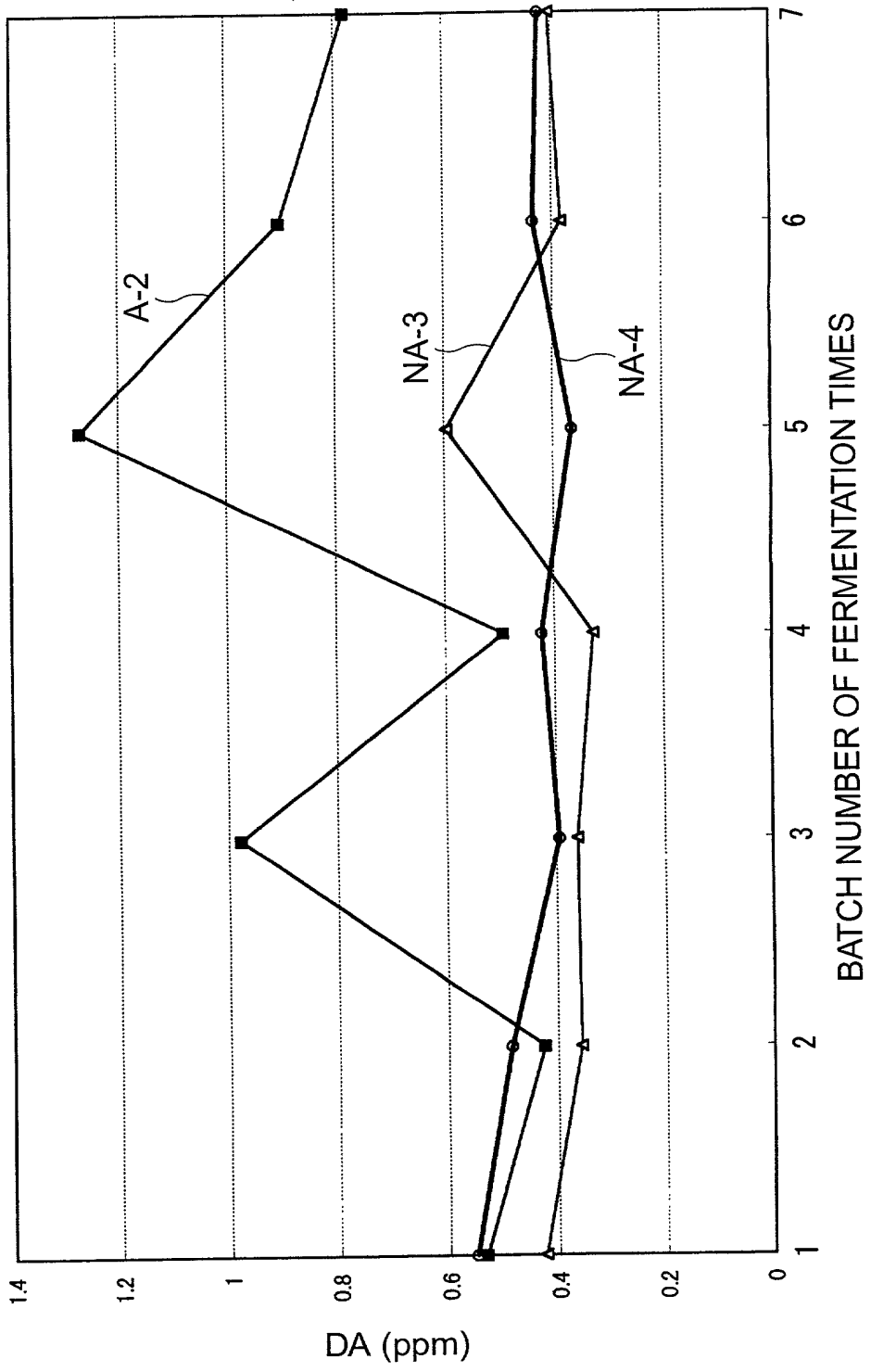
NUMBER OF FLOATING YEAST CELLS
UPON THE END OF PRIMARY FERMENTATION



FOREIGN PATENT DOCUMENT

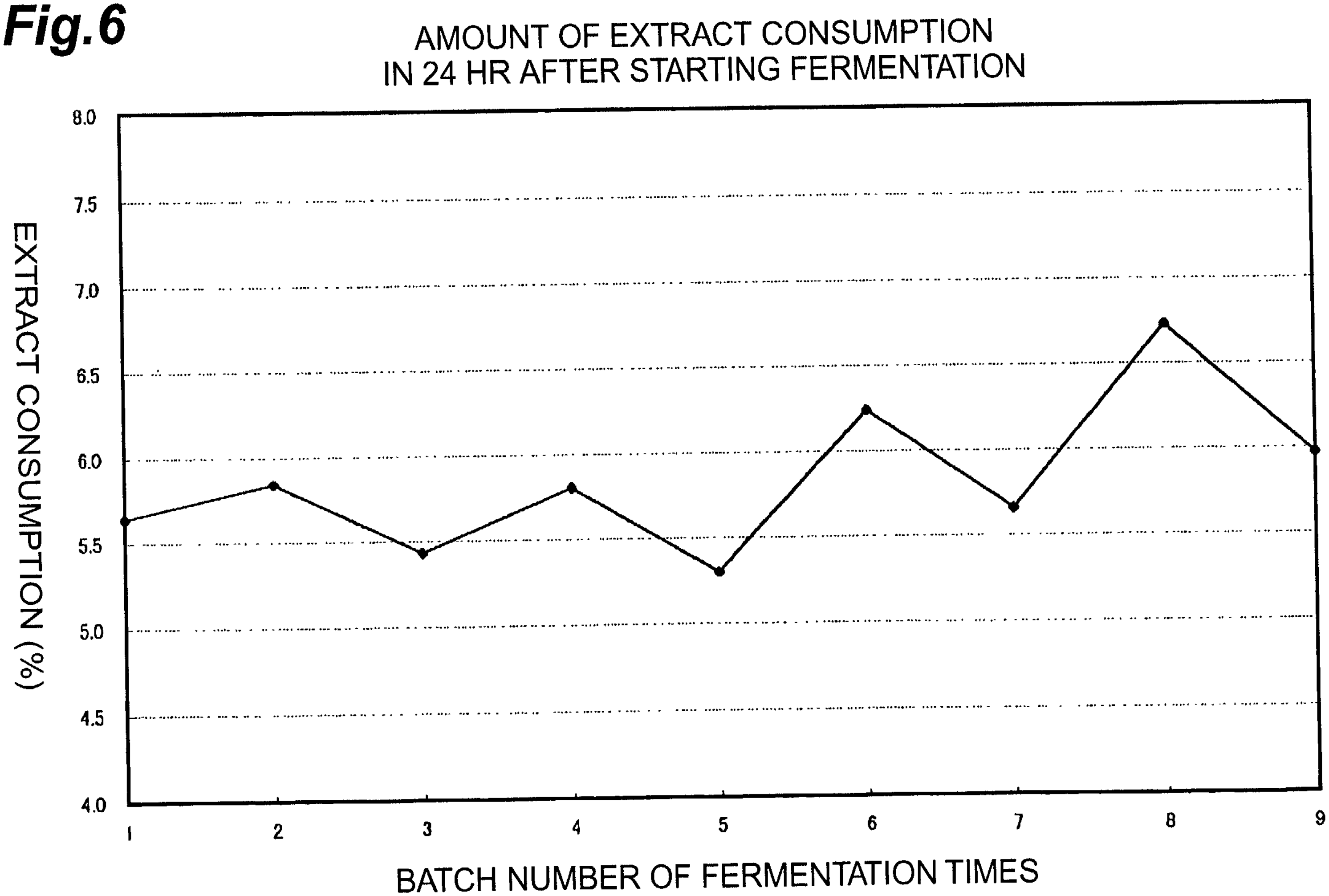
Fig.5

AMOUNT OF DA
UPON THE END OF PRIMARY FERMENTATION



09/763864

Fig.6



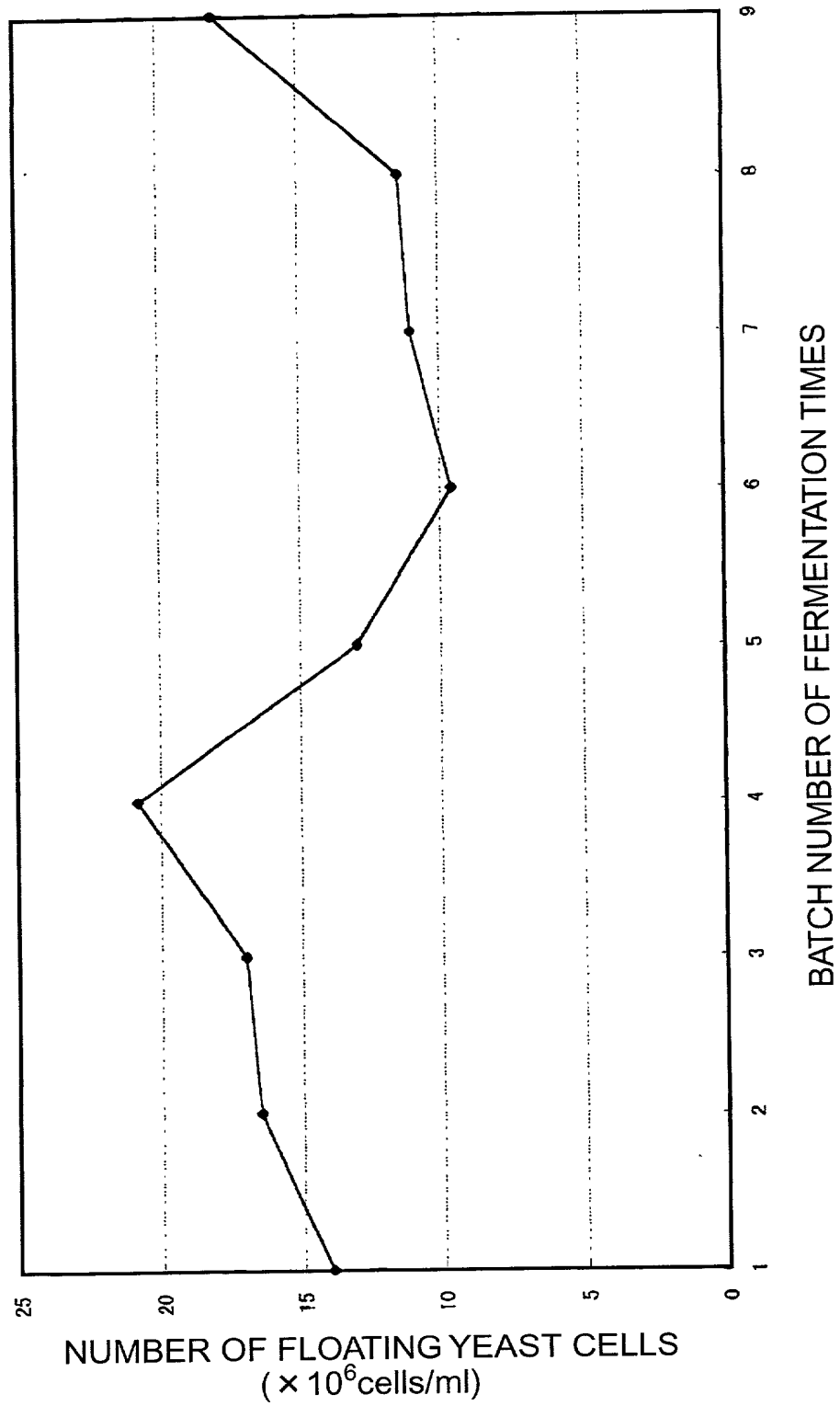
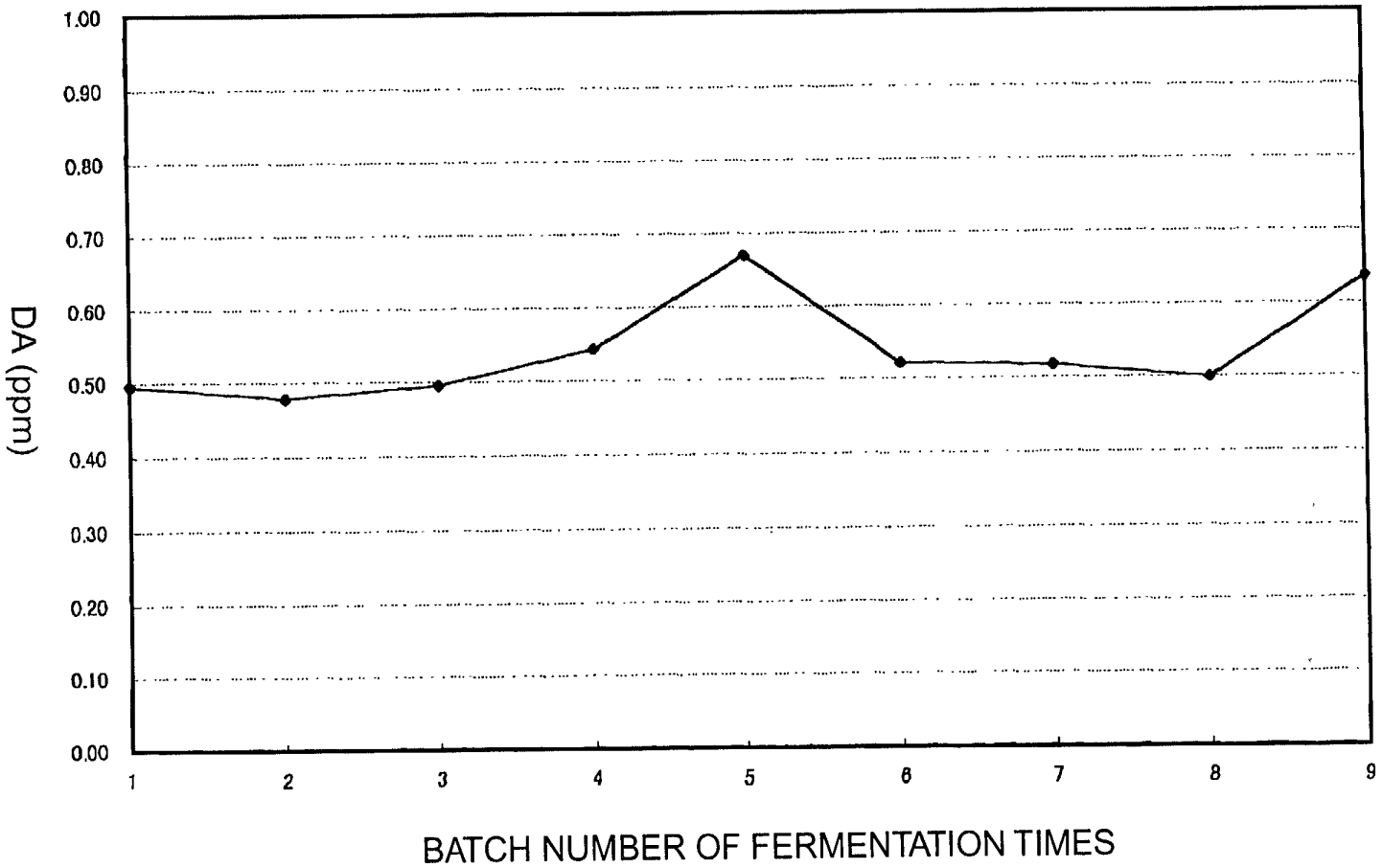

NUMBER OF FLOATING YEAST CELLS
UPON THE END OF PRIMARY FERMENTATION
NUMBER OF FLOATING YEAST CELLS
(×10^6cells/ml)
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
BATCH NUMBER OF FERMENTATION TIMES

Fig.7

TOE250 "438E960

Fig.8

AMOUNT OF DA UPON THE END OF PRIMARY FERMENTATION



OBLON ET AL (703) 413-3000
DOCKET # 2910605 SHEET 8 OF 8
09/763864

Declaration and Power of Attorney For Patent Application

特許出願宣言書及び委任状

Japanese Language Declaration

日本語宣言書

下記の氏名の発明者として、私は以下の通り宣言します。

As a below named inventor, I hereby declare that:

私の住所、私書箱、国籍は下記の私の氏名の後に記載された通りです。

My residence, post office address and citizenship are as stated next to my name.

下記の名称の発明に関して請求範囲に記載され、特許出願している発明内容について、私が最初かつ唯一の発明者（下記の氏名が一つの場合）もしくは最初かつ共同発明者（下記の名称が複数の場合）であると信じています。

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled.

PROCESS FOR PRODUCING FERMENTATION PRODUCT (as amended)

上記発明の明細書は、

☐ 本書に添付されています。

☐ ___月___日に提出され、米国出願番号または特許協定条約国際出願番号を_____とし、
（該当する場合）_____に訂正されました。

the specification of which

☐ is attached hereto.

☒ was filed on 28 February 2001
as United States Application Number or
PCT International Application Number
PCT/JP00/04355 and was amended on
_____ (if applicable).

私は、特許請求範囲を含む上記訂正後の明細書を検討し、内容を理解していることをここに表明します。

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

私は、連邦規則法典第37編第1条56項に定義されるとおり、特許資格の有無について重要な情報を開示する義務があることを認めます。

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

Japanese Language Declaration
(日本語宣言書)

私は、米国法典第35編119条 (a) - (d) 項又は365条 (b) 項に基づき下記の、米国以外の国の少なくとも一ヵ国を指定している特許協力条約365 (a) 項に基づく国際出願、又は外国での特許出願もしくは発明者証の出願についての外国優先権をここに主張するとともに、優先権を主張している、本出願の前に出願された特許または発明者証の外国出願を以下に、枠内をマークすることで、示しています。

I hereby claim foreign priority under Title 35, United States Code, Section 119 (a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)
外国での先行出願

(Number) (番号)	(Country) (国名)	(Day/Month/Year Filed) (出願年月日)	Priority Claimed 優先権主張 Yes はい	No いいえ
11/186117	JAPAN	30 June 1999	☒	☐
			☐	☐

私は、第35編米国法典119条 (e) 項に基づいて下記の米国特許出願規定に記載された権利をここに主張いたします。

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below.

(Application No.) (出願番号)	(Filing Date) (出願日)

私は、下記の米国法典第35編120条に基づいて下記の米国特許出願に記載された権利、又は米国を指定している特許協力条約365条 (c) に基づく権利をここに主張します。また、本出願の各請求範囲の内容が米国法典第35編112条第1項又は特許協力条約で規定された方法で先行する米国特許出願に開示されていない限り、その先行米国出願書提出日以降で本出願書の日本国内または特許協力条約国際提出日までの期間中に入手された、連邦規則法典第37編1条56項で定義された特許資格の有無に関する重要な情報について開示義務があることを認識しています。

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code Section 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of application.

(Application No.) (出願番号)	(Filing Date) (出願日)	(Status: Patented, Pending, Abandoned) (現況：特許許可済、係属中、放棄済)
PCT/JP00/04355	30 June 2000	

私は、私自信の知識に基づいて本宣言書中で私が行なう表明が真実であり、かつ私の入手した情報と私の信じるところに基づく表明が全て真実であると信じていること、さらに故意になされた虚偽の表明及びそれと同等の行為は米国法典第18編第1001条に基づき、罰金または拘禁、もしくはその両方により処罰されること、そしてそのような故意による虚偽の声明を行なえば、出願した、又は既に許可された特許の有効性が失われることを認識し、よってここに上記のごとく宣誓を致します。

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Japanese Language Declaration
（日本語宣言書）

委任状：私は下記の発明者として、本出願に関する一切の手続きを米特許商標局に対して遂行する弁理士または代理人として、下記の者を指名いたします。
（弁護士、または代理人の指名及び登録番号を明記のこと）

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: *(list name and registration number)*

Norman F. Oblon, Reg. No. 24,618; Marvin J. Spivak, Reg. No. 24,913; C. Irvin McClelland, Reg. No. 21,124; Gregory J. Maier, Reg. No. 25,599; Arthur I. Neustadt, Reg. No. 24,854; Richard D. Kelly, Reg. No. 27,757; James D. Hamilton, Reg. No. 28,421; Eckhard H. Kuesters, Reg. No. 28,870; Robert T. Pous, Reg. No. 29,099; Charles L. Gholz, Reg. No. 26,395; William E. Beaumont, Reg. No. 30,996; Jean-Paul Lavalleye, Reg. No. 31,451; Stephen G. Baxter, Reg. No. 32,884; Richard L. Treanor, Reg. No. 36,379; Steven P. Weihrouch, Reg. No. 32,829; John T. Goolkasian, Reg. No. 26,142; Richard L. Chinn, Reg. No. 34,305; Steven E. Lipman, Reg. No. 30,011; Carl E. Schlier, Reg. No. 34,426; James J. Kulbaski, Reg. No. 34,648; Richard A. Neifeld, Reg. No. 35,299; J. Derek Mason, Reg. No. 35,270; Surinder Sachar, Reg. No. 34,423; Jeffrey B. McIntyre, Reg. No. 36,867; William T. Enos, Reg. No. 33,128; Michael E. McCabe, Jr., Reg. No. 37,182; Bradley D. Lytle, Reg. No. 40,073; and Michael R. Casey, Reg. No. 40,294, with full powers of substitution and revocation.

28

書類送付先

Send Correspondence to:

OBLON, SPIVAK, McCLELLAND, MAIER & NEUSTADT, P.C.
FOURTH FLOOR
1755 JEFFERSON DAVIS HIGHWAY
ARLINGTON, VIRGINIA 22202 U.S.A.

直接電話連絡先：（名前及び電話番号）

Direct Telephone Calls to: (name and telephone number)
(703) 413-3000

単独発明者または第一の共同発明者の氏名	Full name of sole or first joint inventor Masahide SATO
発明者の署名　日付	✕Inventor's signature 佐藤雅英　✕Date May 14, 2001
住所	Residence c/o Sapporo Breweries Limited, Brewing Research Laboratories, 10, Okatohme, Yaizu-shi, Shizuoka
国籍	Citizenship 425-0013, JAPAN Citizenship: JAPAN　JPX
郵便の宛先	Post Office Address same as above
第二の共同発明者の氏名	Full name of second joint inventor, if any Shingo UMEMOTO
第二の共同発明者の署名　日付	✕Second joint Inventor's signature 梅本進　✕ Date May 14, 2001
住所	Residence c/o Sapporo Breweries Limited, Brewing Research Laboratories, 10, Okatohme, Yaizu-shi, Shizuoka
国籍	Citizenship 425-0013, JAPAN Citizenship: JAPAN　JPX
郵便の宛先	Post Office Address same as above

（第三以降の共同発明者についても同様に記載し、署名すること）

(Supply similar information and signature for third and subsequent joint inventors.)

6/00